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Dibenzazepinyl ureas as dual NMR and CD probes of helical screw-sense preference in conformationally equilibrating dynamic foldamers

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Vincent Diemer,[a] Julien Maury,[a] Bryden A. F. Le Bailly,[a,b] Simon J. Webb[a,c] and Jonathan Clayden[b*]

Conformationally mobile oligomers with helical structures, or 'dynamic foldamers', may populate a mixture of screw sense conformers whose relative proportion has been used as a means of communicating information on a molecular scale. The dibenzazepinyl urea provides a means of quantifying both the sense and degree of this screw sense preference through a combination of circular dichroism and NMR spectroscopy. The dibenzazepinyl urea probe is synthetically versatile, readily accessible, and easy to introduce to the terminus of an amide or a urea foldamer.

Dynamic foldamers[1] are synthetic oligomers[2] that may switch between alternative well defined conformations as a result of an external influence, for example photochemical irradiation[3-5] or changes in pH.[6] Many dynamic foldamers are helical, and achiral foldamers in this class may be induced to adopt preferentially one of the two possible helical screw senses by intra-[7-10] or intermolecular interaction[6,11] with a chiral 'ligand'. The transmission of a screw-sense preference through a helix,[12,13] which mirrors the conformational transmission of information in biological receptors,[14] has been used as a means of communicating information[15] to a remote reaction site.[5,16] Dynamic helical foldamers built from the achiral quaternary amino acid Aib (aminoisobutyric acid)[17] (Figure 1a) have furthermore been exploited in the membrane phase as mimics of chemo-[18] and photosensitive[3] receptor proteins.

Analysis of the conformational preference of screw-sense invertible dynamic foldamers has relied primarily on NMR[19] and on CD.[20] Both methods have their strengths and limitations when it comes to identifying screw-sense preference, i.e. the prevalence of a left-handed (*M*) or right-handed (*P*) helix. CD reports on the global conformation of a molecule, and is particular useful for distinguishing enantiomeric conformations; NMR can be used to quantify screw-sense preference[19] (though not always to identify the *sense* of the preference, unless an enantiomerically enriched probe[12,21] is present) at precise positions within a foldamer chain, allowing the fine details of conformational induction to be revealed.[13] CD is most powerful when comparisons can be made with related structures (but can therefore be susceptible to systematic error[20,22]); dynamic NMR methods are more generally applicable across several classes of foldamers.[23-25] High resolution NMR requires homogeneous solutions (though solid state NMR can be used to study foldamers in the membrane phase)[3], while CD is more sensitive and can be used to study non-homogeneous systems (although relevant CD bands may be obscured by many solvents).

Given these complementarities between these two techniques, we sought to design a dual-function structural probe that would report structural information about a conformationally mobile dynamic foldamer by both NMR and CD, allowing us to exploit the advantages of both techniques simultaneously.[26] Our solution was to use the dibenzazepine motif (Figure 1b),[27,28] readily incorporated at the terminus of an amide or urea foldamer.

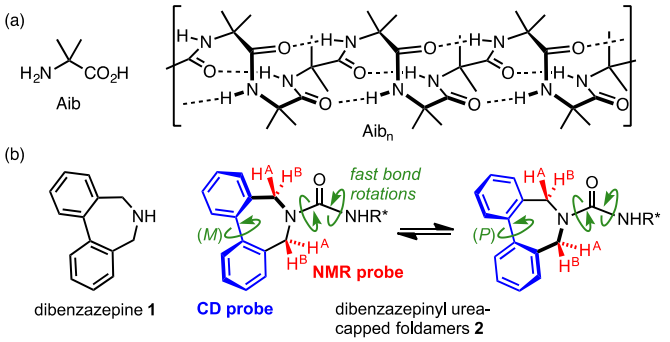

Figure 1: (a) The achiral quaternary amino acid Aib and the 3_{10} helical structure of its olgiomers. (b) Dibenzazepinyl ureas as dual CD and NMR probes, where R* represents the helical oligomer.

Dibenzazepine **1** was made in two steps[29] from commercially available 2,2'-bis(bromomethyl)biphenyl, and coupled to the N terminus of a range of foldamers, as

[a] School of Chemistry, University of Manchester, Oxford Rod, Manchester M13 9PL, UK
[b] School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK.
[c] Manchester Institute of Biotechnology, 131 Princess St., Manchester M1 7DN, UK
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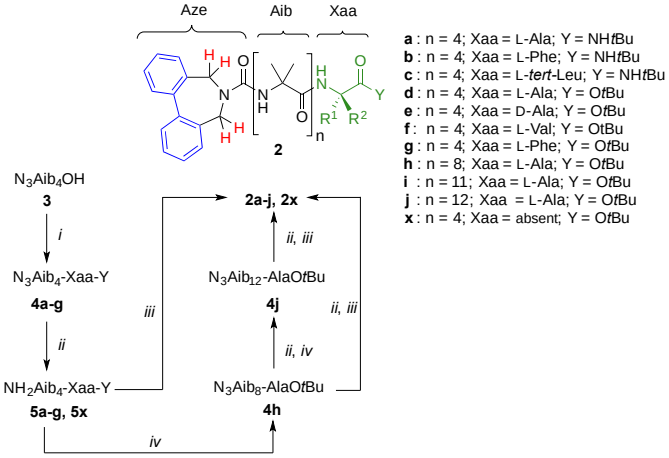
described below, by means of a urea linkage. The resulting urea-capped structures **2** have some important design features. In a chiral environment, the two conformers of the biaryl unit of the dibenzazepine (Figure 1) become differentially populated,[30-34] and hence give a strong, characteristic band in the circular dichroism spectrum in the region of 250 nm.[27,28] Similarly the protons of the two methylene groups of the dibenzazepine ring become diastereotopic, generating an AB system. By linking the dibenzazepinyl group through a urea, rather than an amide or carbamate function, a single AB system is observed: C–N bond rotation in ureas is rapid,[35,36] so the two methylene groups are in fast exchange. Likewise, since interconversion between the two biaryl conformers of the dibenzazepine ring is fast on the NMR timescale, the anisochronicity of the AB system results from the chiral environment experienced by the dibenzazepine probe, rather than from the separate influence of each chiral conformer of the twisted biaryl.[19]

The function of the dibenzazepine probe was explored by ligating it to a series of aliphatic amide foldamers built from Aib[7,20] (Scheme 1). The addition of the amine **1** to the activated carbamates obtained by reaction of peptides **5** with disuccinimidoyl carbonate provided the target compounds **2**. (**2i** (n = 11) was identified as a side product from the synthesis of **2j** (n = 12) from which it was separated by HPLC). Azlactone methodology[12,13] was used to extend the length of Aib oligomers to give the intermediates **4h** (n = 8) and **4j** (n = 12).



a : n = 4; Xaa = L-Ala; Y = NH*t*Bu
b : n = 4; Xaa = L-Phe; Y = NH*t*Bu
c : n = 4; Xaa = L-*tert*-Leu; Y = NH*t*Bu
d : n = 4; Xaa = L-Ala; Y = O*t*Bu
e : n = 4; Xaa = D-Ala; Y = O*t*Bu
f : n = 4; Xaa = L-Val; Y = O*t*Bu
g : n = 4; Xaa = L-Phe; Y = O*t*Bu
h : n = 8; Xaa = L-Ala; Y = O*t*Bu
i : n = 11; Xaa = L-Ala; Y = O*t*Bu
j : n = 12; Xaa = L-Ala; Y = O*t*Bu
x : n = 4; Xaa = absent; Y = O*t*Bu

Scheme 1: Synthesis of the dibenzazepine-capped foldamers. Reagents: i) NH_2-Xaa-Y, HOBt, EDC or EDC·HCl, DIPEA or TEA, CH_2Cl_2, RT; ii) H_2, Pd/C, MeOH; iii) DSC, CH_2Cl_2, RT followed by DIPEA, HCl.**1**, CH_3CN or DMF, RT; iv) **3**, EDC·HCl, RT followed by MeCN, reflux. TEA = triethylamine; DIPEA = N,N-diisopropylethylamine, EDC = *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide, HOBt = 1-hydroxybenzotriazole, DSC = *N,N*′-disuccinimidyl carbonate

These Aib foldamers **2** of various lengths carry at their N terminus the dibenzazepine probe, and at their C terminus a chiral residue capable of inducing a screw sense preference in the intervening oligo-Aib chain. CD spectra for this series of compounds in methanol, illustrated in Figure 2, exhibit (in addition to the bands associated with the amide groups at 208 and 220 nm) an intense and characteristic band at 250 nm correlated to the *M* or *P* torsion at the aryl-aryl bond of the probe. Foldamers **2a-c** and **2e**, which have *P* screw sense in the foldamer helix, exhibit a positive band; **2d**, **2f-h** and **2j** have an *M* screw sense and exhibit a negative band. No CD signal was detected for the 'control' achiral foldamer **2x**. The dibenzazepine probe is evidently sensitive to the asymmetric environment promoted by the chiral amino acid residue located at the other end of the peptide and this asymmetric induction occurs in spite of large spatial separations between the dynamic sensor and the chiral inducer. In the case of the 13-mer **2j**, this distance is estimated to 24 Å.[37]

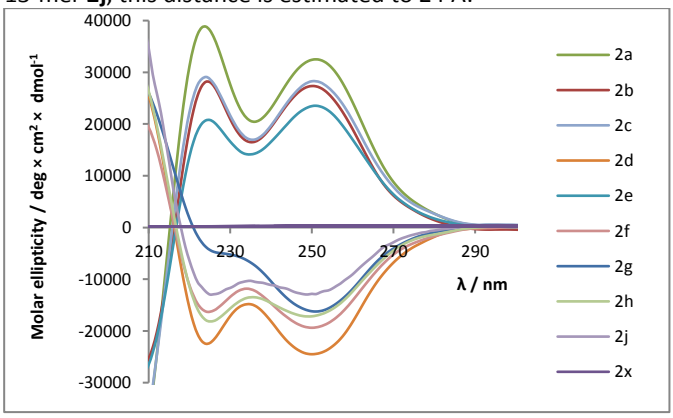


Figure 2 : CD spectra of peptides **2a-h**, **2j** and **2x** in MeOH.

The intensity of the CD signal was also used to quantify the induced screw sense preference at the N terminus of the foldamer. The *P* and *M* conformers of Aib oligomers interconvert rapidly[38] through a low-energy pathway that involves a transient, short-lived and mobile screw-sense reversal.[13,39] The screw sense preference induced by a covalently attached chiral residue can be expressed as a ratio of these *P* and *M* conformers or, more conveniently, as a 'helical excess',[7] {[*P*] – [M]} / {[P] + [M]}. As shown in Figure 3, the molar ellipticities of compounds **2a-h**, **2j** and **2x** measured at 250 nm display a linear correlation with the helical excesses either determined by ^{13}C NMR at the N terminus of equivalent structures[7] or extrapolated in the case of longer peptides (**2h** and **2j**) using an exponential decay model.[7,13] This proportionality indicates that the biased equilibrium between the left- and right-handed helical conformations induces the same ratio of conformers (*M* or *P*) in the dynamic dibenzazepine probe, with an *M* helix inducing *P* torsion in the probe and hence a negative band[28] and *vice versa* for a *P* helix. These results provide a simple way to quantify the conformational preference of the Aib helix, with the helical excess in methanol being given by the formula

$$\text{h.e. (\%)} = 100 \times \theta_{250nm} \text{ (deg cm}^2 \text{ dmol}^{-1})/41100$$

Biaryl-derived structures, in the form of the amino acid Bip,[40] have been used to identify the absolute helix screw sense of peptides, but not to quantify screw sense ratios in this way. As well as being easier to introduce than Bip, dibenzazepinyl ureas exhibit an additional advantageous feature in the form of the signal arising from the CH_2 groups in their NMR spectra (Figure 4).

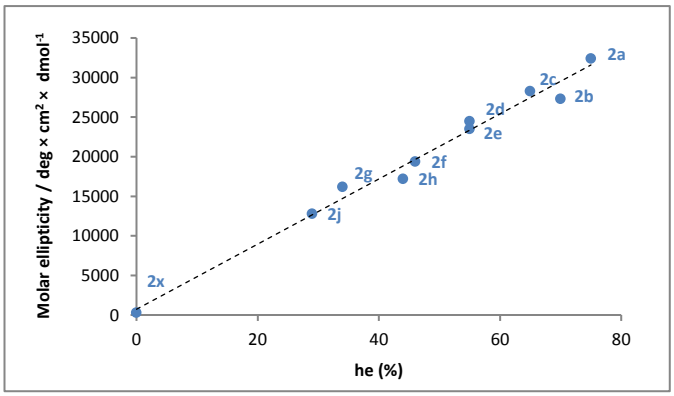


Figure 3: Comparison of the absolute molar ellipticity θ measured at 250 nm with previously reported or calculated helical excess in MeOH.

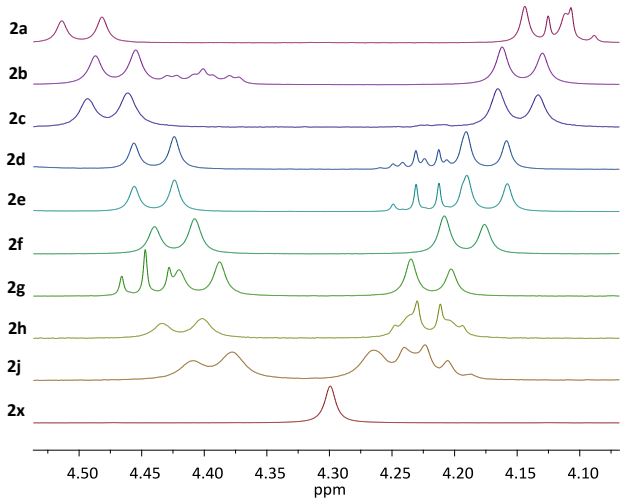


Figure 4: Portion of the ^{1}H NMR in CD_3OH of **2a-h**, **2j** and **2x**, showing the anisochronicity Δδ of the methylene proton of the dibenzazepine probe.

Provided the chiral influence inducing a screw sense preference in the system is remote from the dibenzazepine probe, the chemical shift separation between H^A and H^B will be proportional to the helical excess at the site of the probe induced by the remote chiral residue.[19] As shown Figure 5, the chemical shift separation of the two anisochronous peaks in compounds **2a-j** and **2x** is proportional to the helical excess at the N terminus of the oligomer induced by the chiral motif at the C terminus, both in methanol and in THF. Helical excess can thus be calculated from Δδ using the formula

$$\text{h.e. (\%)} = 100 \times \Delta\delta \text{ (ppb)}/ 480 \text{ (in methanol)}$$

or

$$\text{h.e. (\%)} = 100 \times \Delta\delta \text{ (ppb)}/ 496 \text{ (in THF)}$$

The NMR and CD spectra of the dibenzazepine-capped foldamers thus both provide a means of measuring helical excess, but only CD reports the absolute sense of the helical preference. NMR is also insensitive to the effect of enantiomeric excess, reporting only the conformational preference of each individual molecule. This is graphically illustrated in Figure 6, which shows the effect on both CD and NMR of varying the relative proportions of the enantiomeric foldamers **2d** and **2e**.

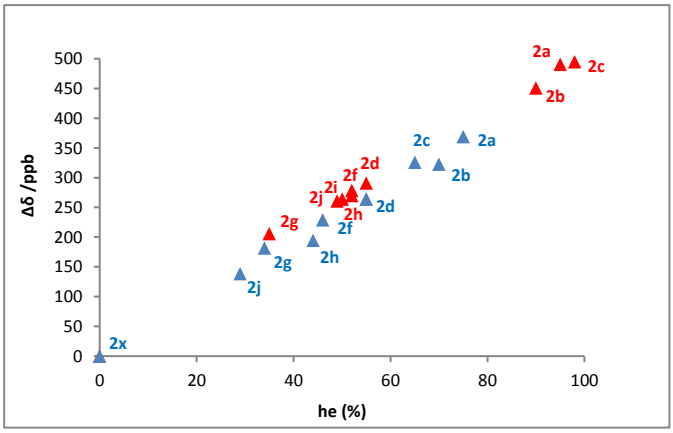


Figure 5: Correlation of the anisochronicity Δδ in the benzylic protons of the azepine probe with helical excess in CD_3OH (blue) and in THF-d_8 (red).

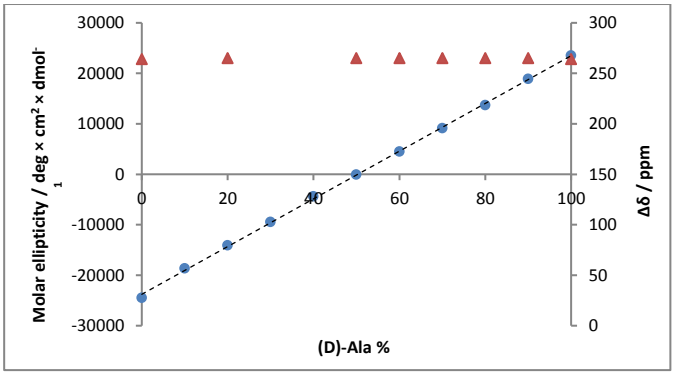
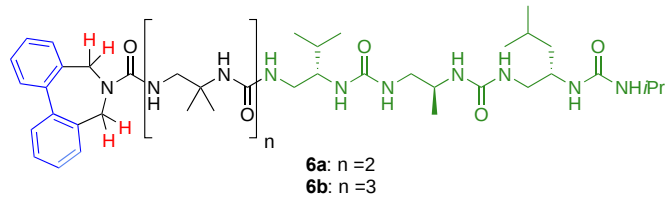


Figure 6: Variation of the anisochronicity Δδ of the methylene groups of the dibenzazepine probe (Δ) and the molar ellipticity θ measured at 250 nm of the chromophore (○) with the enantiomeric purity of the AlaO*t*Bu motif.

The applicability of the dibenzazepine probe to other foldamer classes was also demonstrated by ligating it to the terminus of two oligoureas **6** in which a chiral screw-sense inducing domain comprising three chiral diamines[41,42] is ligated to either two or three achiral diamines (Figure 7). Previous studies on these foldamers[24] suggested that the right-handed screw sense preference of the chiral domain persists through the achiral domain, but that the degree of control decays rather rapidly. This conclusion was supported by the CD spectra of **6a** and **6b**. The degree of screw sense control drops by more than 50% with the addition of an additional achiral diamine residue. The anisochronicity of the methylene groups similarly decreased from 74 ppb in **6a** to ca. 27 ppb in **6b**.

In summary, a dibenzazepinyl urea functions as a dual NMR and chiroptical probe that is readily available and easy to introduce to the terminus of amide and urea foldamers. Combining data from NMR and CD provides information on the sign and value of screw sense preference.



6a: n =2
6b: n =3

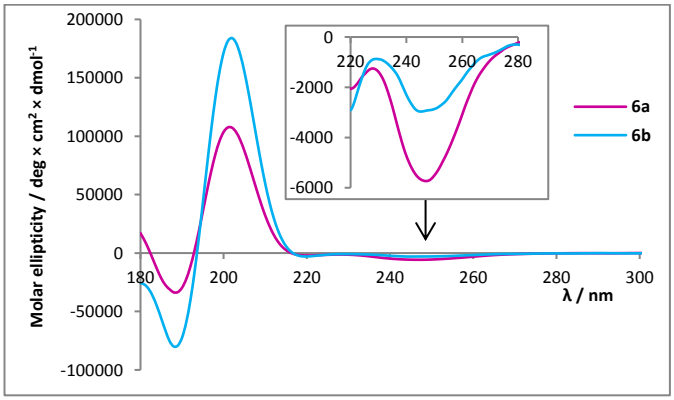


Figure 7: The dibenzazepinyl probe capping a urea foldamer and the change in molar ellipticity on adding an additional achiral residue.

Acknowledgements

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Dibenzazepinyl ureas act as probes to allow conformational analysis of screw sense preference in dynamic foldamers by both NMR and CD.